(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number
WO 01/05435 A2

(51) International Patent Classification⁷: A61K 48/00

(21) International Application Number: PCT/CA00/00853

(22) International Filing Date: 19 July 2000 (19.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/144,495 19 July 1999 (19.07.1999) US

(71) Applicants *(for all designated States except US)*: **THE UNIVERSITY OF BRITISH COLUMBIA** [CA/CA]; University-Industry Liaison Office, IRC Building, Room 331, 2194 Health Sciences Mall, Vancouver, British Columbia V6T 1Z3 (CA). **MIYAKE, Hideaki** [JP/JP]; #505-6-2-15 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017 (JP).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **GLEAVE, Martin** [CA/CA]; 4693 Drummond Drive, Vancouver, British Columbia V6R 1E8 (CA).

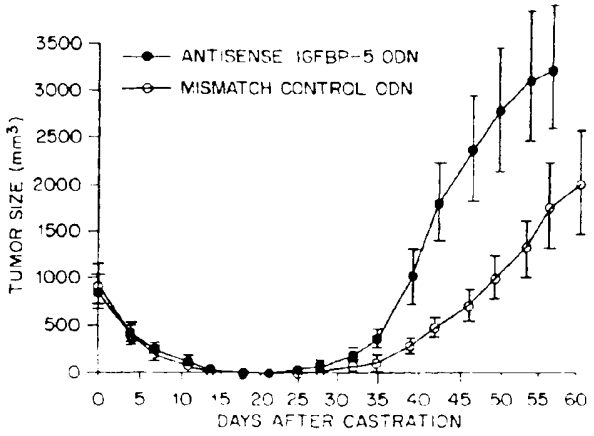
(74) Agents: **KINGWELL, Brian, G.** et al.; Smart & Biggar, Box 11560, Vancouver Centre, 650 West Georgia Street, Suite 2200, Vancouver, British Columbia V6B 4N8 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,

[Continued on next page]

(54) Title: ANTISENSE THERAPY FOR HORMONE-REGULATED TUMORS



(57) Abstract: A method is provided for treating hormone regulated tumors (for example, breast and prostatic tumors) in mammals, including humans, by administration of an antisense ODN which is complementary to a portion of the gene encoding IGFBP-5. Using the Shionogi tumor model *in vitro* and *in vivo*, the administration of such an ODN was shown to reduce proliferation of tumor cells, and also to delay the progression to androgen independence. Thus, treatment of prostate cancer in mammals, including humans, and delay of the progression of prostate tumors to androgen independence is accomplished by administering to the mammal a therapeutically effective amount of an antisense oligodeoxynucleotide which is complementary to a portion of the nucleic acid sequence encoding IGFBP-5 and which hybridizes with such a sequence to inhibit expression of IGFBP-5. Specific antisense ODN's which are suitable for use in the method are GACCACGCTGATCACCAT (Seq. ID. No. 1), which is derived from the murine gene sequence, and CGCGGTGAGCAACACCAT (Seq. ID. No. 3) and AGGTCATGCAGCAGCCGC (Seq. ID No 4), which are derived

WO 01/05435 A2

IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- *Without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

ANTISENSE THERAPY FOR HORMONE-REGULATED TUMORS

This application claims priority from US Provisional Application No. 60/144,495 filed July 19, 1999, which is incorporated herein by reference.

Background of the Invention

This application relates to the treatment of hormone-regulated tumors (for example, breast and prostate tumors), making use of an antisense oligonucleotide that binds to insulin-like growth factor binding protein (IGFBP)-5.

Prostate cancer is the most common cancer that affects men, and the second leading cause of cancer deaths in men in the Western world. Because prostate cancer is an androgen-sensitive tumor, androgen withdrawal, for example via castration, is utilized in some therapeutic regimens for patients with advanced prostate cancer. Androgen withdrawal leads to extensive apoptosis in the prostate tumor, and hence to a regression of the disease. However, castration-induced apoptosis is not complete, and a progression of surviving tumor cells to androgen-independence ultimately occurs. This progression is the main obstacle to improving survival and quality of life, and efforts have therefore been made to target androgen-independent cells. These efforts have focused on non-hormonal therapies targeted against androgen-independent tumor cells, however thusfar no non-hormonal agent has improved survival. Oh et al., *J. Urol* 160: 1220-1229 (1998) Alternative approaches are therefore indicated.

Insulin-like growth factor (IGF)-I and IGF-II are potent mitogens for many normal and malignant cells. Accumulating evidence suggests that IGFs play an important role in the pathophysiology of prostatic disease and breast cancer. Boudon et al., *J. Clin. Endocrin. Metab.* 81: 612-617 (1996); Angelloz-Nicoud et al., *Endocrinology* 136: 5485-5492 (1995); Nickerson et al., *Endocrinology* 139: 807-810 (1998); Figueroa et al., *J. Urol.* 159: 1379-1383 (1998).

The biological response to IGF's is regulated by various factors, including IGFBPs. To date, six IGFBPs have been identified whose function is believed to involve modulation of the biological actions of the IGFs through high affinity interactions. Rajaram et al., *Endocrin. Rev.* 18: 801-813 (1997). However, some

evidence suggests biological activity for IGFBPs that are independent of IGFs, *Id.*, Andress et al., *J. Biol. Chem.* 267: 22467-22472 (1992); Oh et al., *J. Biol. Chem.* 268: 14964-14971 (1993), and both stimulatory and inhibitory effects of IGFBPs on cell proliferation have been reported under various experimental conditions. Andress et al., *supra*, Elgin et al., *Proc. Nat'l. Acad. Sci. (USA)*, 84, 3254-3258 (1987); Huynh et al., *J. Biol. Chem.* 271: 1016-1021 (1996); Damon et al., *Endocrinology* 139: 3456-3464 (1998). Thus, the precise function role of IGFBPs remains controversial. Because of this, while the reported results implicate IGF in prostate and breast cancer, they do not clearly suggest a therapeutic approach based upon this involvement.

The present invention utilizes antisense oligodeoxynucleotides (ODNs) targeted to IGFBP-5 as a treatment for prostate and breast cancer. Antisense ODNs are chemically modified stretches of single-stranded DNA that are complementary to mRNA regions of a target gene, and thereby effectively inhibit gene expression by forming RNA/DNA duplexes. Figueroa, et al., *J. Urol., 159:* 1379-1383 (1998). Phosphorothioate ODNs are stabilized to resist nuclease digestion by substituting one of the nonbridging phosphoryl oxygen of DNA with a sulfur. Recently, several antisense ODNs specifically targeted against genes involved in neoplastic progression have been evaluated both *in vitro* and *in vivo*, and demonstrated the efficacy of antisense strategy as potential therapeutic agents. Monia, et al. *Nature Med. 2:* 668-675 (1996.); Cucco, et al., *Cancer Res. 56:* 4332-4337 (1996); Ziegler, et al., *J. Natl. Cancer Inst. 89:* 1027-1036 (1997); Jansen, et al., *Nature Med. 4:* 232-234 (1998).

Summary of the Invention

The present invention provides a method for treating hormone-regulated tumors (for example, breast and prostatic tumors) in mammals, including humans, by administration of an antisense ODN which is complementary to a portion of the gene encoding IGFBP-5. Using the Shionogi tumor model *in vitro* and *in vivo*, the administration of such an ODN was shown to reduce proliferation of tumor cells, and also to delay the progression to androgen independence. Thus, in accordance with the invention we provide methods for treatment of prostate cancer in mammals, including humans, and for delaying the progression of prostate tumors to androgen

independence comprising the step of administering to the mammal a therapeutically effective amount of an antisense oligodeoxynucleotide which is complementary to a portion of the nucleic acid sequence encoding IGFBP-5 and which hybridizes with such a sequence to inhibit expression of IGFBP-5. Specific antisense ODN's which are suitable for use in the method are GACCACGCTGATCACCAT (Seq. ID. No. 1), which is derived from the murine gene sequence, and CGCGGTGAGCAACACCAT (Seq. ID. No. 3) and AGGTCATGCAGCAGCCGC (Seq. ID No 4), which are derived from the human gene sequence.

Brief Description of The Figures

Fig. 1 shows the effects of antisense IGFBP-5 ODN in decreasing the regrowth of tumor cells following surgical androgen withdrawal;

Fig. 2 shows the reduction in IGFBP-5 mRNA following treatment with antisense IGFBP-5 ODN *in vivo*;

Fig. 3 shows the dosage-dependence of the reduction in IGFBP-5 mRNA following treatment with antisense IGFBP-5 ODN *in vitro*;

Fig. 4 shows the number of cells present following treatment with antisense IGFBP-5 ODN as a function of time;

Fig. 5 shows the number of cells present following treatment with antisense IGFBP-5 ODN as a function of concentration;

Fig.. 6 shows the proportion of dead cells in a sample treated with antisense IGFBP-5 ODN;

Fig. 7 shows the effects of antisense IGFBP-5 ODN in relation to IGF-1 and anti-IGF-1 antibody;

Fig. 8 shows flow cytometry results for cells treated with antisense IGFBP-5 ODN;

Fig. 9 shows a schematic representation of the nucleotide sequence for human IGBFP-5, with the locations of 10 antisense ODN's indicated; and

Fig. 10 shows a effect of each of the 10 antisense ODN's indicated in Fig. 9 on IGBFP-5 mRNA levels.

Detailed Description of The Invention

The present invention provides a method for delaying the progression of hormone-regulated (prostatic or breast) tumor cells to hormone (e.g. androgen or estrogen) independence, a therapeutic method for the treatment of individuals, including humans, suffering from hormone regulated cancers, such as breast or prostate cancer, and therapeutic agents effective for use in such methods. In addition, the compositions of the invention can be used to inhibit or delay the growth and metastatic progression of prostate, breast and other IGF-1 sensitive tumors in bone. The therapeutic method of the invention will most commonly be used in the treatment of individuals with advanced breast or prostate cancer.

In accordance with a first embodiment of the invention, the progression of androgen-sensitive prostatic cancer cells to androgen-independence can be delayed by inhibiting the expression of IGFBP-5 by the cells. Experiments were performed *in vitro* and *in vivo* in the Shionogi tumor model. The Shionogi tumor model is a xenograft of an androgen-dependent mouse mammary carcinmoa that grows subcutaneously in male syngeneic hosts. Shionogi tumor cells are highly tumorigenic and locally invasive. The cells have been shown to respond to androgen withdrawal in a manner which mimics the observed behavior of prostatic tumor cells, and have been accepted as a valid model for prostate cancer in humans. (Bruchovsky et al., *Cancer Res.* 50: 2275-2282 (1990); Rennie et al., *Cancer res.* 48: 6309-6312 (1988); Bruchovsky et al., *Cell* 13: 272-280 (1978); Gleave et al., in *Genitourinary Oncology*, pp. 367-378, Lange et al. eds., Lippencott (1997); Gleave et al., *J. Urol.* 157: 1727-1730 (1997); Bruchovsky et al., *The Prostate* 6: 13-21 (1996). Thus, androgen withdrawal precipitates apoptosis and tumor regression in a highly reproducible manner. Further, changes in expression of peptides such as TRPM-2 and Bcl-2 in human prostate cancer following castration and during progression to androgen-independence are similar to those observed in Shionogi tumor cells. Because of these similarities, the Shionogi tumor model mimics human prostate cancer and provides a very useful model for the evaluation of the ability of compounds to delay the onset of androgen-independence. Despite complete tumor regression after castration, rapidly growing androgen-independent Shionogi tumors invariably recur after one month,

which provides a reliable end point to evaluate agents which can delay the progression to androgen-independence.

In the study leading to the present invention, we initially characterized the changes of IGFBPs expression in the Shionogi tumor model after castration and during AI progression. Northern blot analyses were used to characterize changes in IGFBPs mRNA expression in AD intact tumors before castration, regressing tumors 4 and 7 days after castration, and AI recurrent tumors 28 days after castration. Various patterns of changes in IGFBP-2, -3, -4, and -5 mRNA expression were observed. IGFBP-1 and IGFBP-6 mRNAs are undetectable in the Shionogi tumor model. Of the IGFBPs expressed in Shionogi tumors, the most dramatic changes in expression were observed with IGFBP-5. Despite undetectable levels in AD intact tumors, IGFBP-5 expression is highly upregulated after castration, and remains highly expressed in AI tumors. The pattern of IGFBP-5 upregulation in the Shionogi tumor model during AI progression is similar to that in rat prostate (Angelloz-Nicoud, *supra)*, and human prostate cancer (Figueroa, *supra*), and therefore supports use of this model to evaluate the effect of adjuvant antisense IGFBP-5 therapy on progression to androgen-independence.

To study the functional significance of this upregulation, we tested the effects of antisense IGFBP-5 ODN on IGF-1 mediated cell growth both *in vitro* and *in vivo* using the Shionogi tumor model. These tests were carried out using an antisense ODN directed against the murine IGFBP-5 gene. These experiments showed that phosphorothioate antisense IGFBP-5 ODN corresponding to the mouse *IGFBP-5* translation initiation site inhibited expression of IGFBP-5 mRNA in a dose-dependent manner. Sequence specificity was confirmed using a 2-base IGFBP-5 mismatch ODN, which had no effects on IGFBP-5 mRNA expression in Shionogi tumor cells. Furthermore, we demonstrated that antisense IGFBP-5 ODN decreased IGFBP-5 expression in a target specific manner; that is, the expression of other mRNAs, including IGFBP-2, -3, and -4, were not affected by antisense IGFBP-5 ODN treatment.

Antisense IGFBP-5 ODN inhibits cell proliferation and induces cell cycle arrest in Shionogi tumor cells in a time- and dose-dependent manner. Antisense

IGFBP-5 ODN treatment does not appear to induce apoptosis either *in vitro* or *in vivo*, which suggests that antisense IGFBP-5 ODN activity occurs via inhibition of cell proliferation rather than induction of apoptosis. Further, it was observed that the growth-inhibitory effects of antisense IGFBP-5 ODN can be reversed by exogenous IGF-1 and that antisense IGFBP-5 ODN treatment caused no additional inhibition of cell proliferation when IGF-1 activity was neutralized by anti-IGF-1 antibodies. We also found that antisense IGFBP-5 ODN inhibited MAPK activity, that this inhibition could also be reversed by exogenous IGF-1, and that antisense IGFBP-5 ODN had no independent inhibitory effect on MAPK activity when IGF-1 was neutralized by anti-IGF-1 antibodies. Collectively, these findings demonstrate that antisense IGFBP-5 ODN inhibited the cell proliferation, at least in part, through an IGF-I-dependent mechanism involving inactivation of MAPK.

Based on this *in vitro* data, we hypothesized that targeting IGFBP-5 upregulation precipitated by androgen using antisense strategy might inhibit progression to androgen-independence. In our *in vivo* experiments, administration of antisense IGFBP-5 ODN after castration delayed time to AI progression and inhibited AI recurrent tumor growth. Consistent with our *in vitro* treatments, *in vivo* treatment of mice bearing Shionogi tumors with antisense IGFBP-5 ODN also inhibited the IGFBP-5 mRNA expression. These findings illustrate that *in vivo* systemic administration of ODN can result in sequence specific down-regulation of a target gene in tumor cells.

Although insulin-like growth factor (IGF) binding protein-5 (IGFBP-5) is highly up-regulated in normal and malignant prostate tissues after androgen withdrawal, its functional role in castration-induced apoptosis and androgen-independent progression remains undefined. To analyze the functional significance of IGFBP-5 overexpression in IGF-I-mediated mitogenesis and progression to androgen-independence, IGFBP-5-overexpressing human androgen-dependent LNCaP prostate cancer cells were generated by stable transfection. The growth rates of IGFBP-5 transfected LNCaP cells were significantly faster compared to either the parental or vector-only transfected LNCaP cells in both the presence and absence of dihydrotestosterone. IGFBP-5-induced increases in LNCaP cell proliferation occurs

through both IGF-I-dependent and -independent pathways, with corresponding increases in the cyclin D1 mRNA expression and the fraction of cells in S + G2/M phases of the cell cycle. Changes in Akt/protein kinase B (PKB), a downstream component of phosphatidylinositol 3'-kinase (PI3K) pathway, in the LNCaP sublines also paralleled changes in their growth rates. Although treatment with a PI3K inhibitor induced apoptosis in both control and IGFBP-5-overexpressing LNCaP cells, this PI3K inhibitor-induced apoptosis was prevented by exogenous IGF-I treatment only in IGFBP-5 transfectants, suggesting that IGFBP-5 overexpression can potentiate the antiapoptotic effects of IGF-I. Furthermore, tumor growth and serum PSA levels increased several fold faster in mice bearing IGFBP-5-transfected LNCaP tumors after castration despite having similar tumor incidence and tumor growth rates with controls when grown in intact mice before castration. Collectively, these data suggest that IGFBP-5 overexpression in prostate cancer cells after castration is an adaptive cell survival mechanism that helps potentiate the antiapoptotic and mitogenic effects of IGF-I, thereby accelerating progression to androgen-independence through activation of the PI3K-Akt/PKB signaling pathway.

A rational strategy to delay AI progression should be based on molecular mechanisms and would target the adaptive changes in gene expression precipitated by androgen withdrawal, rather than the conventional approach of treating patients with established hormone refractory disease. Integration and appropriate timing of combination therapies, based on biological mechanism of progression and castration-induced changes in gene expression, may provide means to inhibit AI progression in a major way. The present study provides direct evidence to support a functional role for IGFBP-5 in AI progression, and that reduction of IGFBP-5 gene expression using antisense IGFBP-5 ODN delays recurrence and growth of AI tumors.

The treatment of the present invention can be used individually. However, the antisense ODNs are preferably utilized in combination with other therapies, that result in androgen-withdrawal. Thus, in accordance a further aspect of with the invention, therapeutic treatment of individuals, including human individuals, suffering from prostate cancer is achieved by initiating androgen-withdrawal to induce apoptotic cell death of prostatic tumor cells in the individual, and administering to the individual a

composition effective to inhibit expression of IGBFP-5 by the tumor cells, thereby delaying the progression of prostatic tumor cells to an androgen-independent state in an individual. In view of the expression of IGFBP-5 in bone, IGF-1 and IGFBP-5 mediated tumor cells growth may also play a substantial role in promoting growth of IGF-1 sensitive metastatic tumor cells in bone. This growth can be prevented through the use of the antisense IGBFP-5 ODN of the invention, thus inhibiting or delaying the progression of metastatic disease.

Initiation of androgen withdrawal may be accomplished via surgical (removal of both testicles) or medical (drug-induced suppression of testosterone) castration, which is currently indicated for treatment of prostate cancer. Medical castration can be achieved with various regimens, including LHRH agents and antiandrogens. Gleave et al. *CMAJ* 160: 225-232 (1999). Intermittent therapy in which reversible androgen withdrawal is effected is described in Gleave et al. *Eur. Urol.* 34, Supp 3: 37-41 91998). Hormone withdrawal in the case of breast cancer can be achieved through drug therapy with anti-estrogenics such as tamoxifen.

The inhibition of IGBFP-5 expression may be transient, and should occur following androgen withdrawal. In humans, this means that inhibition of expression should be effective starting within weeks of androgen withdrawal and extending for about 3 to 6 months. This may require multiple doses to accomplish. It will be appreciated, however, that the period of time may be more prolonged, starting before castration and expending for substantial time afterwards without departing from the scope of the invention.

The ODN used in the tests described above and in the examples below (Seq. ID. No. 1) is complementary to a portion of the murine IGFBP-5 gene overlaps with the translation initiation site. Other ODN species might also be employed, including somewhat long or somewhat shorter ODN species (for example in the range of 15 to 30 nt) that overlap with the translation initiation site, and ODN species that overlap with the translation termination site. Intermediate ODN's may also be effective, and can be screened for their ability to provide adequate levels of IGFBP-5 inhibition using the expression assay described in the examples. In selecting the antisense ODN for use, it is desirable to avoid substantial complementarity with other IGFBPs, since

inhibition of expression of these other proteins might lead to undesirable side effects. The nucleic acid sequence of mouse IGFBP-5 from which such ODN can be derived is given by SEQ ID No. 13.

To apply the invention in other mammals, including humans, therapeutic antisense ODNs are derived from the corresponding locations in the IGFBP-5 gene of the target species. For example, in the case of humans, the sequence of the IGFBP-5 gene is known from Kiefer et al., *Biochem. Biophys Res. Commun.* 176: 219 (1991), Accession No. M65062 for human and James et al., *J. Biol. Chem.* 258: 22305 (1993), Accession No. L12447 for mouse. Fig. 10 shows the locations of several antisense ODN's which were tested for the ability to inhibit expression of IGFBP-5 in humans has the sequence given by Seq. ID. No. 3. This ODN overlaps with the translation initiation site of human IGFBP-5. As in the case of the mouse model, other human therapeutic antisense ODNs may be employed, including somewhat long or somewhat shorter ODN species (for example in the range of 15 to 30 nt) that overlap with or are located near the translation initiation site (for example SEQ ID No. 4), and ODN species that overlap with the translation termination site (for example SEQ ID No. 10). Intermediate ODN's may also be effective, and can be screened for their ability to provide adequate levels of IGFBP-5 inhibition using the expression assay described in the examples. In selecting the antisense ODN for use, it is desirable to avoid substantial complementarity with other IGFBPs, since inhibition of expression of these other proteins might lead to undesirable side effects. The complete sequence of human IGFBP-5 from which other antisense ODN can be derived is given by SEQ ID No. 14. SEQ ID Nos. 15-66 list additional antisense ODN sequences designed from the sequence of human IGFBP-5.

The ODNs employed may be modified to increase the stability of the ODN *in vivo*. For example, the ODNs may be employed as phosphorothioate derivatives (replacement of a non-bridging phosphoryl oxyegns atoms with a sulfur atom) which have increased resistance to nuclease digestion. Increased ODN stability can also be achieved using molecules with 2-methoxyethyl substituted backbones.

Administration of antisense ODNs can be carried out using the various mechanisms known in the art, including naked administration and administration in

pharmaceutically acceptable carriers. For example, lipid carriers for antisense delivery are described in US Patents Nos. 5,855,911 and 5,417,978 which are incorporated herein by reference. In general, the antisense is administered by intravenous, intraperitoneal, subcutaneous or oral routes.

The amount of antisense ODN administered is one effective to inhibit the expression of IGBFP-5 in breast cancer or prostatic cells. It will be appreciated that this amount will vary both with the effectiveness of the antisense ODN employed, and with the nature of any carrier used. The determination of appropriate amounts for any given composition is within the skill in the art, through standard series of tests designed to assess appropriate therapeutic levels. .

The method for treating prostate or breast cancer in accordance with the invention may further include administration of chemotherapy agents and/or additional antisense ODNs directed at different targets. For example, conventional chemotherapy agents such as taxol (paclitaxel or docitaxel) and mitoxanthrone may be used. Similarly, combinations of antisense IGFBP-5 ODN with other antisense species such as antisense Bcl-2 ODN or TRPM-2 ODN may be used.

The invention will now be further described with reference to the following, non-limiting examples.

EXAMPLE 1

Shionogi tumor model experiments were performed using cells from the Toronto subline of transplantable SC-115 AD mouse mammary carcinoma, and maintained in Dulbecco's modified Eagle medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 5% heat-inactivated fetal calf serum. For *in vivo* studies, approximately 5 X 10^6 cells of the Shionogi carcinoma were injected subcutaneously in adult male DD/S strain mice. When the Shionogi tumors became 1 to 2 cm in diameter, usually 2 to 3 weeks after injection, castration was performed through an abdominal incision under methoxyflurane anesthesia. Details of the maintenance of mice, tumor stock and operative procedures have been previously described. Bruchovsky et al., *Cancer Res.* 5); 2275-2282 (1990); Rennie et al., *Cancer Res.* 48: 6309-6312 (1988); Bruchovsky et al., *Cell* 13: 272-280 (1978).

Mice were randomly selected for treatment with murine phosphorothioate antisense IGFBP-5 ODN (Seq. ID No. 1) or a mismatch control having the sequence GACCACGCTCATGACCAT (Seq. ID No. 12) which is two bases different in sequence from the antisense IGFBP-5 ODN. Each experimental group consisted of 8 mice. Beginning the day of castration, 15 mg/kg of antisense IGFBP-5 or mismatch control ODN were injected intraperitoneally once daily into each mouse for 50 days. Tumor volume was measured twice weekly, and calculated by the formula length X width X depth X 0.5236. Gleave, *Cancer Res.* 52: 1598-1605 (1992). Data points were reported as average tumor volumes ± standard deviation.

The results of this study are shown in Fig.1. Antisense IGFBP-5 ODN treatment delayed recurrence of AI tumors compared to mismatch control ODN treatment. Although AI tumors recurred in all mice in both groups during an observation period of 60 days post-castration, median time to first palpable AI recurrence increased by 25% from 28 to 35 days in mice treated with antisense IGFBP-5 vs mismatch control ODN. Mice required sacrifice when tumor mass increased above 3 cm^3 or 10% of body weight. Growth of recurrent AI tumors was substantially inhibited in antisense IGFBP-5 ODN treatment group compared to the mismatch control ODN group. Time to sacrifice of mice was significantly prolonged in the antisense IGFBP-5 ODN treatment group, all mice required sacrifice in mismatch ODN group after a median of 53 days compared to only 1 of 8 mice in antisense IGFBP-5 ODN treatment group after 60 days ($p<0.05$).

Example 2

To examine the effects of *in vivo* ODN treatment on levels of IGFBP-5 mRNA, Northern blot analysis was performed on Shionogi tumor tissue from mice. Mice were treated daily, beginning the day of castration, with 15 mg/kg of antisense IGFBP-5 ODN (n=3) or the mismatch control (n=3) by intraperitoneal injection. On the fourth day after castration, tumor tissues were harvested and analyzed by Northern blot for IGFBP-5 mRNA. Antisense IGFBP-5 ODN resulted in a 61% reduction in IGFBP-5 mRNA levels in Shionogi tumors compared to mismatch control ODN treated tumors. (Fig. 2).

EXAMPLE 3

The sequence selectivity of the antisense IGFBP-5 ODN (Seq. ID. No. 1) was confirmed by comparing expression levels of IGFBP-5 mRNA in Shionogi tumor cells maintained *in vitro*, after treatment with varying levels of antisense IGFBP-5 ODN (Seq. ID No. 1) or a mismatch control (Seq. ID. No. 12). To facilitate uptake of the ODNs into the cells, the ODNs were formulated in a cationic lipid carrier (Lipofectin™, (Life Technologies, Inc.)). Cells were treated twice over a period of two days using the following protocol. Cells were preincubated for 20 minutes with 4 μg/ml of lipofectin in serum free OPTI-MEM™ (Life Technologies, Inc.) and then incubated with the medium containing the selected concentration of ODN and lipofectin for four hours. The medium was then replaced with the standard culture medium described in Example 1.

The amount of IGFBP-5 mRNA in the cells was evaluated using Northern blot analysis. As shown in Fig. 3, daily treatment of Shionogi cells with antisense IGFBP-5 ODN (Seq. ID No. 1) at levels of 50, 100, 500 or 1000 nM reduced IGFBP-5 mRNA levels in a dose dependent manner by 0, 7, 54 or 83%, respectively. In contrast, IGFBP-5 mRNA levels were not affected by the mismatch ODN (Seq. ID. No. 3) at any of the employed concentrations. Thus, the affect of antisense IGFBP-5 ODN is apparently sequence specific.

To further analyze the specificity of antisense IGFBP-5 ODN, Northern blotting was performed after treatment of Shionogi tumor cells with 1 μM antisense IGFBP-5 ODN (Seq. ID. No. 1) to quantify changes in expression other IGFBP (IGFBP-2, -3, and -4) genes, which share significant sequence homology with IGFBP-5. Antisense IGFBP-5 ODN markedly reduced IGFBP-5 mRNA expression, but no effects were observed on IGFBP-2, -3, and -4 expression levels. Collectively these data demonstrate that antisense IGFBP-5 ODN used in these studies induce sequence-specific, gene-specific, and dose-dependent downregulation of its target gene.

EXAMPLE 4

To determine the effects of antisense IGFBP-5 ODN on cell proliferation, we treated Shionogi tumor cells once daily with either 1 μM antisense IGFBP-5 or

mismatch control ODN for 2 days, and determined cell number over a 72 h period. Antisense IGFBP-5 ODN treatment of cells resulted in significant inhibition of Shionogi tumor cell proliferation over this 72 h, whereas cell growth was not influenced by treatment with mismatch control ODN (Fig. 4).

The effects of antisense IGFBP-5 ODN on cell proliferation were also found to be dose-dependent over a concentration range between 100 and 1000 nM (Fig. 5). These antiproliferative effects were directly correlated with the degree of IGFBP-5 mRNA reduction in Shionogi tumor cells by antisense IGFBP-5 ODN. In contrast, no significant effects were observed on cell proliferation with mismatch control ODN at any of the employed concentrations.

To exclude the possibility that antisense IGFBP-5 ODN acted as a cell death factor through induction of apoptosis, the number of live and dead cells were counted after antisense or mismatch IGFBP-5 ODN treatment. Live and dead Shionogi cells from each subculture were counted using trypan blue 48 h after ODN treatment, and the ratio of dead/total cells was calculated. Each assay was performed in triplicate. The observed ratio of dead cells to total cell number of antisense IGFBP-5 ODN-treated cells was not significantly different from that of mismatch control ODN-treated cells (Fig. 6). Hence, differences in cell number after antisense IGFBP-5 ODN treatment are not the result of enhanced apoptosis

EXAMPLE 5

To analyze the relationship between IGFBP-5 and IGF-I in the regulation of Shionogi tumor cell growth, the effects of antisense IGFBP-5 ODN treatment on Shionogi tumor cell proliferation with anti-IGF-I antibodies and/or recombinant IGF-I were evaluated. In a first experiment, the *in vitro* effects of antisense IGFBP-5 ODN, anti-IGF-I antibody (Upstate Biotechnology, Lake Placid, NY), and/or recombinant IGF-I (Sigma Chemical Co., St. Louis, MO) on growth of Shionogi tumor cells were assessed by the MTT assay as described previously Miyake, et al., *Oncogene 16:* 933-943 (1998). . Briefly, 1 X 10^4 cells were seeded in each well of 96-well microtiter plates and allowed to attach overnight. Cells were then treated once daily with various concentrations of ODN for 2 days in the media containing 5 nM recombinant IGF-I or

10 μg/ml anti-IGF-I antibody. 48 h after ODN treatment, 20 μl of 5 mg/ml MTT (Sigma Chemical Co.) in PBS was added to each well, followed by incubation for 4 h at 37°C. The formazan crystals were then dissolved in dimethyl sulfoxide. The optical density was determined with a microculture plate reader (Becton Dickinson Labware, Lincoln Park, NJ) at 540 nm. Absorbance values were normalized to the values obtained for the vehicle-treated cells to determine the percent of survival. Each assay was performed in triplicate.

As shown in Fig. 7, recombinant IGF-I increased Shionogi tumor cell proliferation, while anti IGF-1 neutralizing antibodies inhibited Shionogi cell growth by 60%. Furthermore, inhibition of cell proliferation by antisense IGFBP-5 ODN could be reversed by exogenous recombinant IGF-I treatment. Addition of antisense IGFBP-5 ODN with anti IGF-1 neutralizing antibodies did not add to the inhibitory effects of anti IGF-1 neutralizing antibodies alone. Collectively, these findings support an enhancing and IGF-I-dependent effect of IGFBP-5 on the cell proliferation.

Because MAPK is one of the most potent pathways for IGF-I signal transduction, we measured the effects of antisense IGFBP-5 and anti IGF-1 neutralizing antibodies on MAPK activity in Shionogi tumor cells. Mitogen-activeted protein kinase (MAPK) activity was measured using a MAP Kinase Assay Kit (New England Biolabs, Beverly, MA). Briefly, the cells were washed with PBS, lysed in lysis buffer, sonicated, and microcentrifuged for 20 min at 4°C. The supernatants was incubated with 1:100-diluted anti-phospho- MAPK antibody for 4 h. Protein A-agarose beads were then added and incubated for another 3 h. The pellets were washed twice with ice-cold lysis buffer and twice with kinase buffer. The pellets were incubated with 100 mM ATP and 20mg/ml Elk1 fusion protein, a substrate of MAPK, for 30 min at 30°C. Samples were boiled, separated by electrophoresis through a 10% SDS-polyacrylamide gel, and transferred to polyvinylidene difluoride membranes. The membranes were incubated for 1 h at room temperature in blocking buffer, and then probed with 1:1000-diluted anti-phospho-Elk1 antibody. After wash, the membranes were incubated with a 1:1000-diluted horseradish peroxidase-conjugated anti-rabbit immunogrobulin. The immunoreactivity of phosphorylated Elk1 was determined using an ECL chemiluminescence kit.

Observed changes in MAPK activity mirrored changes in cell proliferation induced by these agents; that is, antisense IGFBP-5 ODN reduced MAPK activity, this antisense IGFBP-5-induced decrease in MAPK activity effect could be reversed by recombinant IGF-I, and antisense IGFBP-5 had no additional inhibitory effect on MAPK activity when the mitogenic effects of IGF-I were neutralized by anti-IGF-I antibodies.

Example 6

To examine effects of changes in IGFBP-5 expression levels on cell cycle regulation, flow cytometric analysis was performed in Shionogi tumor cells. The flow cytometric analysis of propidium iodide-stained nuclei was performed as described previously Miyake, *supra*. Briefly, Shionogi tumor cells were plated at a density of 5 X 10^6 cells in 6-cm dishes, and treated as described above. The cells were trypsinized 48 h after ODN treatment, washed twice with PBS, and fixed in 70% ethanol for 5 h at 4°C. The fixed cells were washed twice with PBS, incubated with 1 µg /ml RNaseA (Sigma Chemical Co.) for 1 h at 37°C and stained with 5 µg/ml propidium iodide (Sigma Chemical Co.) for 1 h at room temperature. The stained cells were analyzed for relative DNA content on a FACScan TM (Becton Dickinson Labware).

As shown in Fig. 8, decreases in IGFBP-5 levels induced by antisense IGFBP-5 ODN treatment resulted in G1 cell cycle arrest, thereby reducing the fraction of cells in the S + G2/M phases by more than 50% compared to mismatch control ODN treatment.

Example 7

To identify appropriate antisense IGFBP-5 ODN sequences for use in human therapy, antisense ODN sequences directed against 10 different sites of the human IGFBP-5 gene (Fig. 9, Seq. ID Nos. 2-11) were synthesized and tested for their ability to decrease IGFBP-5 gene expression in human prostate cancer PC3 cells and LNCaP/T1 (LNCaP cells stably transfected to overexpress IGFBP-5) in *in vitro* cell culture. The results are summarized in Fig. 10. As shown, Seq. ID Nos. 3, 4 and 10 are active for reduction of IGFBP-5 expression, with Seq. ID No. 3 having the greatest potency. These three sequence overlap with or are immediatley adjacent to the translation initiation or termination sites.

Example 8

Metastatic prostate and breast cancer frequently invade bony tissue. Treatment of such metastases is very difficult, and progression of the cancer into the bone generally indicates a poor prognosis for long term survival. Thus, it would be desirable to have a methodology for inhibiting or delaying this progression. It was logical to assume that since IGF-1 and IGFBP-5 are important factors for growth of IGF-1 sensitive cancer, including in particular prostate and breast cancer, that the presence of high levels of IGFBP-5 in bone could be an important mechanism for promoting the growth and progression of metastatic lesions. Accordingly, Western analysis was performed on samples of primary human bone tissue obtained from patients suffering from metastatic prostate cancer. This experiment confirmed the presence of high levels of IGFBP-5 in bone. Inhibition of these levels using antisense IGFBP-5 ODN in accordance with the invention should provide an effective therapy for inhibiting or delaying the progression of metastatic lesions in the bone.

CLAIMS

1. A composition for treatment of hormone-regulated cancer comprising an antisense oligonucleotide which inhibits expression of IGFBP-5 by hormone-regulated tumor cells.

2. The composition of claim 1, wherein the antisense oligonucleotide is complementary to a region of IGFBP-5 mRNA including the translation initiation or termination site.

3. The composition of claim 2, wherein the antisense oligonucleotide comprises a series of contiguous bases as set forth in SEQ ID No. 3, 4 or 10.

4. The composition of claim 2 or 3, wherein the antisense oligonucleotide has a length of from 15 to 30 nucleotides.

5. Use of a composition according to any of claims 1-4 in formulating a pharmaceutical composition for delaying progression of hormone-regulated tumor cells to an androgen- independent state by treating hormone-sensitive tumor cells with an antisense oligonucleotide which inhibits expression of IGFBP-5 by the tumor cells.

6. The use of claim 5, wherein the tumor cells are prostatic tumor cells.

7. The use of claim 5, wherein the tumor cells are breast cancer cells.

8. Use of a composition according to any of claims 1-4 in formulating a pharmaceutical composition for treating a hormone-responsive cancer in an individual suffering from hormone-responsive cancer, wherein the pharmaceutical composition is administered to the individual after initiation of hormone-withdrawal to induce apoptotic cell death of hormone-responsive

cancer cells in the individual, and thereby delays the progression of hormone-responsive cancer cells to a hormone-independent state in the individual.

9. The use of claim 8, wherein the hormone-responsive cancer is prostate cancer.

10. Use of a composition in accordance with any of claims 1-4 in formulating a pharmaceutical composition for inhibiting or delaying metastatic boney progression of an IGF-1 sensitive tumor in a mamma by administration of the composition to the mammal in an amount effective to inhibit expression of IGFBP-5 by the hormone-responsive cancer cells, thereby inhibiting or delaying metastatic boney progression of the tumor.

11. The use of claim 10, wherein the IGF-1 sensitive tumor is a prostate cancer.

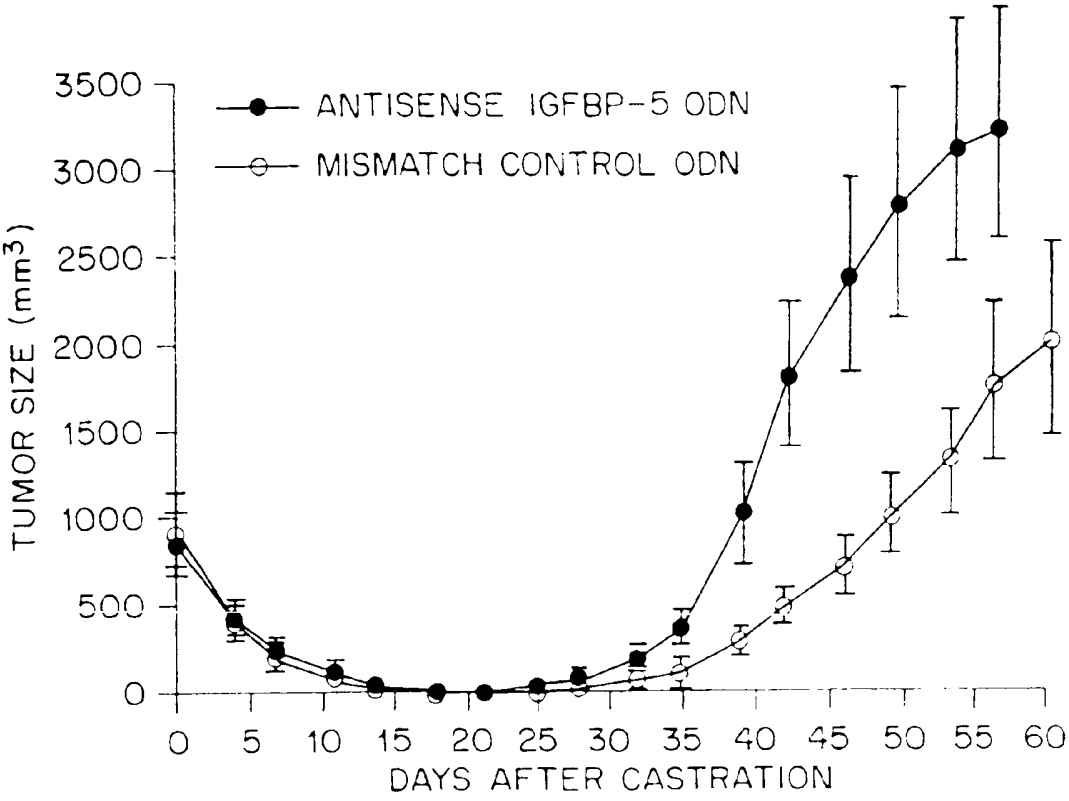

ANTISENSE IGFBP-5 ODN
MISMATCH CONTROL ODN
TUMOR SIZE (mm3)
3500
3000
2500
2000
1500
1000
500
0
0 5 10 15 20 25 30 35 40 45 50 55 60
DAYS AFTER CASTRATION

FIG. 1

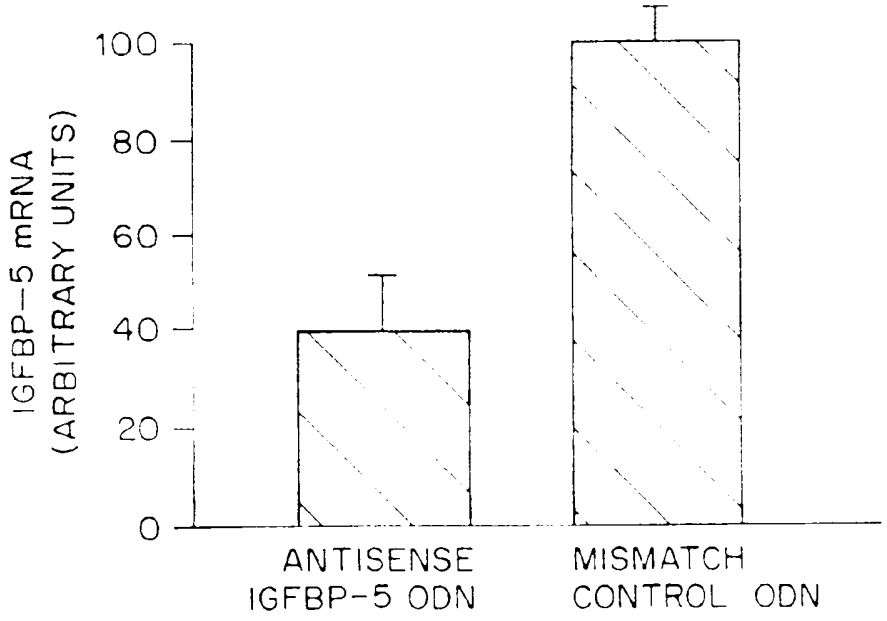

IGFBP-5 mRNA
(ARBITRARY UNITS)
100
80
60
40
20
0
ANTISENSE
IGFBP-5 ODN
MISMATCH
CONTROL ODN

FIG. 2

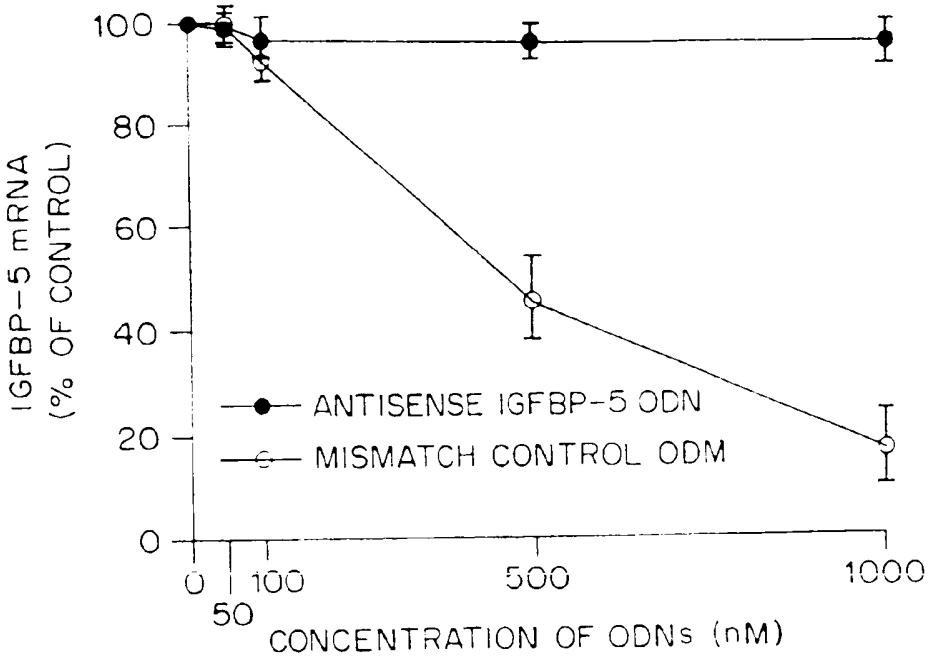

100
80
60
40
20
0
IGFBP-5 mRNA
(% OF CONTROL)
ANTISENSE IGFBP-5 ODN
MISMATCH CONTROL ODM
0
50
100
500
1000
CONCENTRATION OF ODNs (nM)

FIG. 3

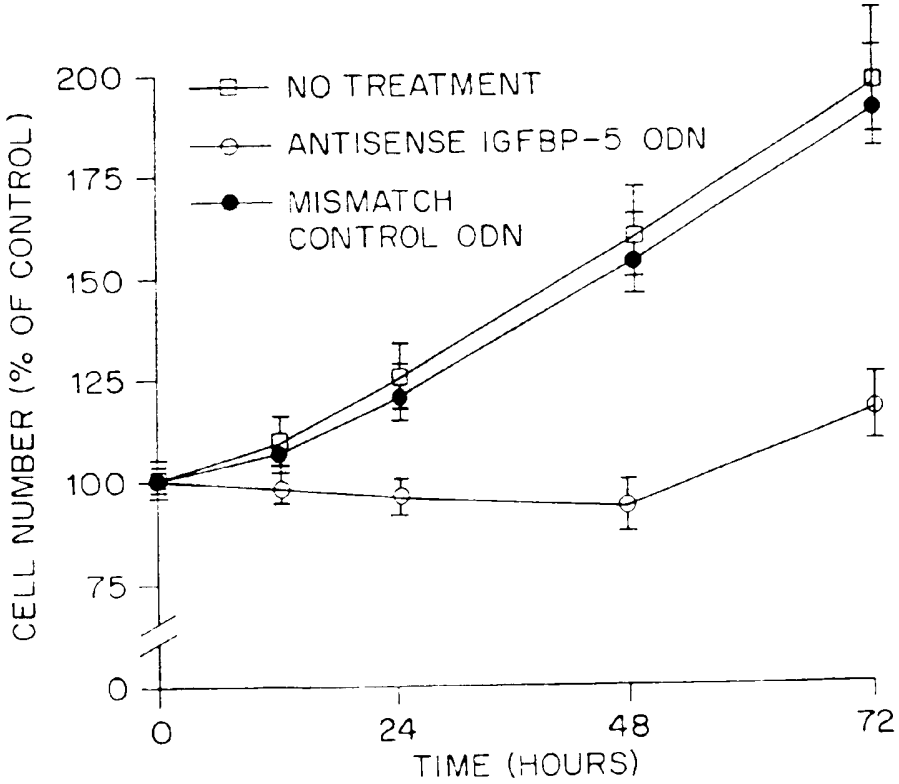

200
175
150
125
100
75
0
CELL NUMBER (% OF CONTROL)
NO TREATMENT
ANTISENSE IGFBP-5 ODN
MISMATCH
CONTROL ODN
0
24
48
72
TIME (HOURS)

FIG. 4

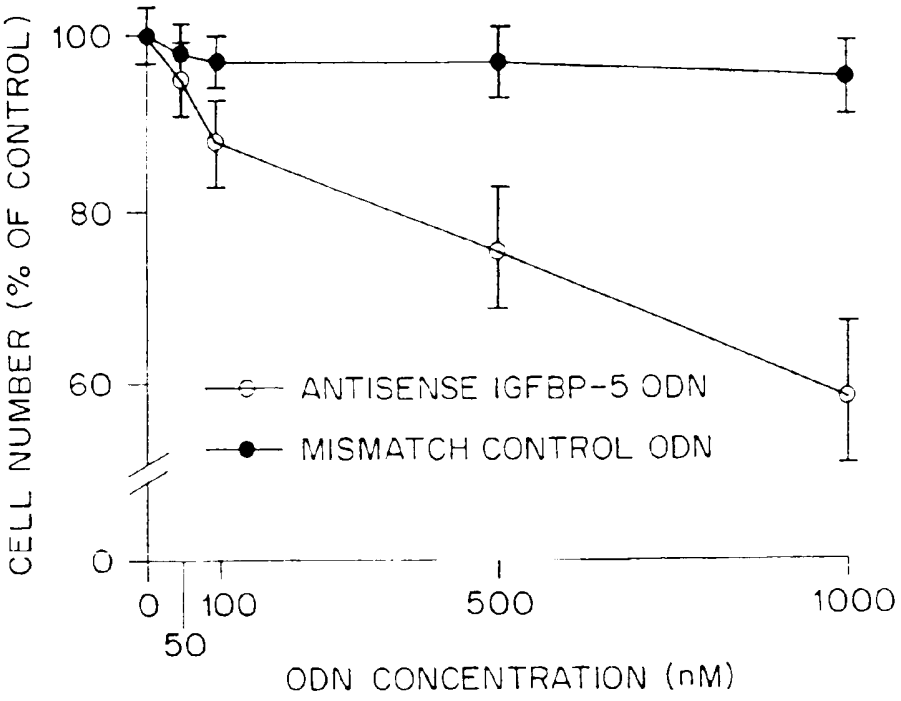

CELL NUMBER (% OF CONTROL)
100
80
60
0
ANTISENSE IGFBP-5 ODN
MISMATCH CONTROL ODN
0
50
100
500
1000
ODN CONCENTRATION (nM)

FIG. 5

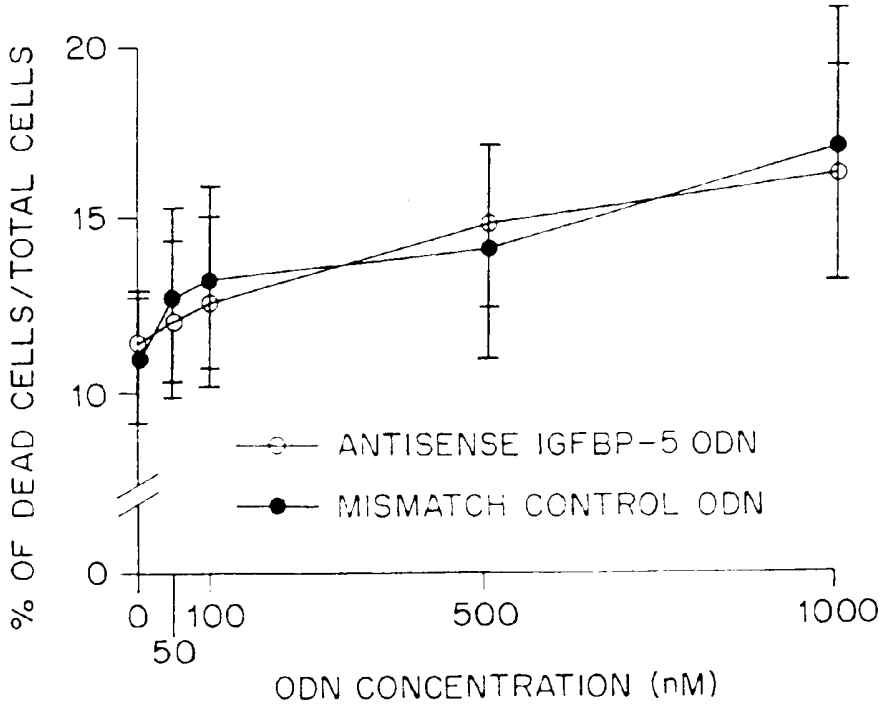

% OF DEAD CELLS/TOTAL CELLS
20
15
10
0
ANTISENSE IGFBP-5 ODN
MISMATCH CONTROL ODN
0
50
100
500
1000
ODN CONCENTRATION (nM)

FIG. 6

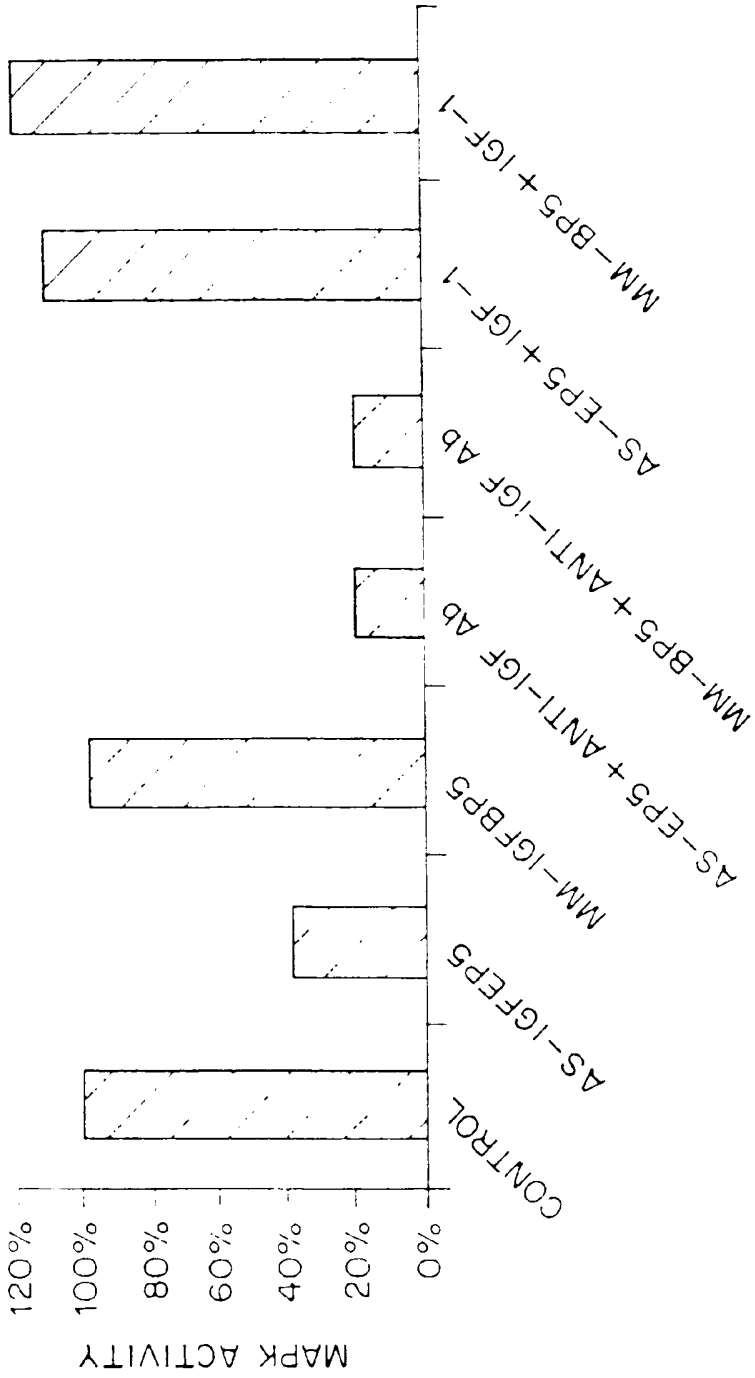
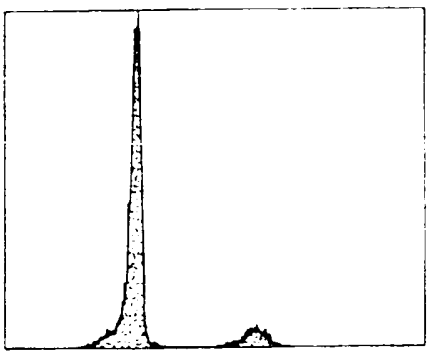
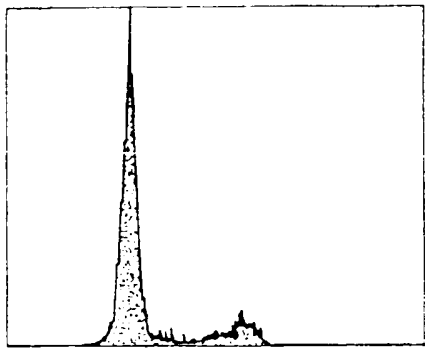

MAPK ACTIVITY
120%
100%
80%
60%
40%
20%
0%
CONTROL
AS-IGFEP5
MM-IGFBP5
AS-EP5+ANTI-IGF Ab
MM-BP5+ANTI-IGF Ab
AS-EP5+IGF-1
MM-BP5+IGF-1

FIG. 7



1μM ANTISENSE IGFBP-5
ODN TREATMENT



1μM MISMATCH CONTROL
ODN TREATMENT

FIG. 8

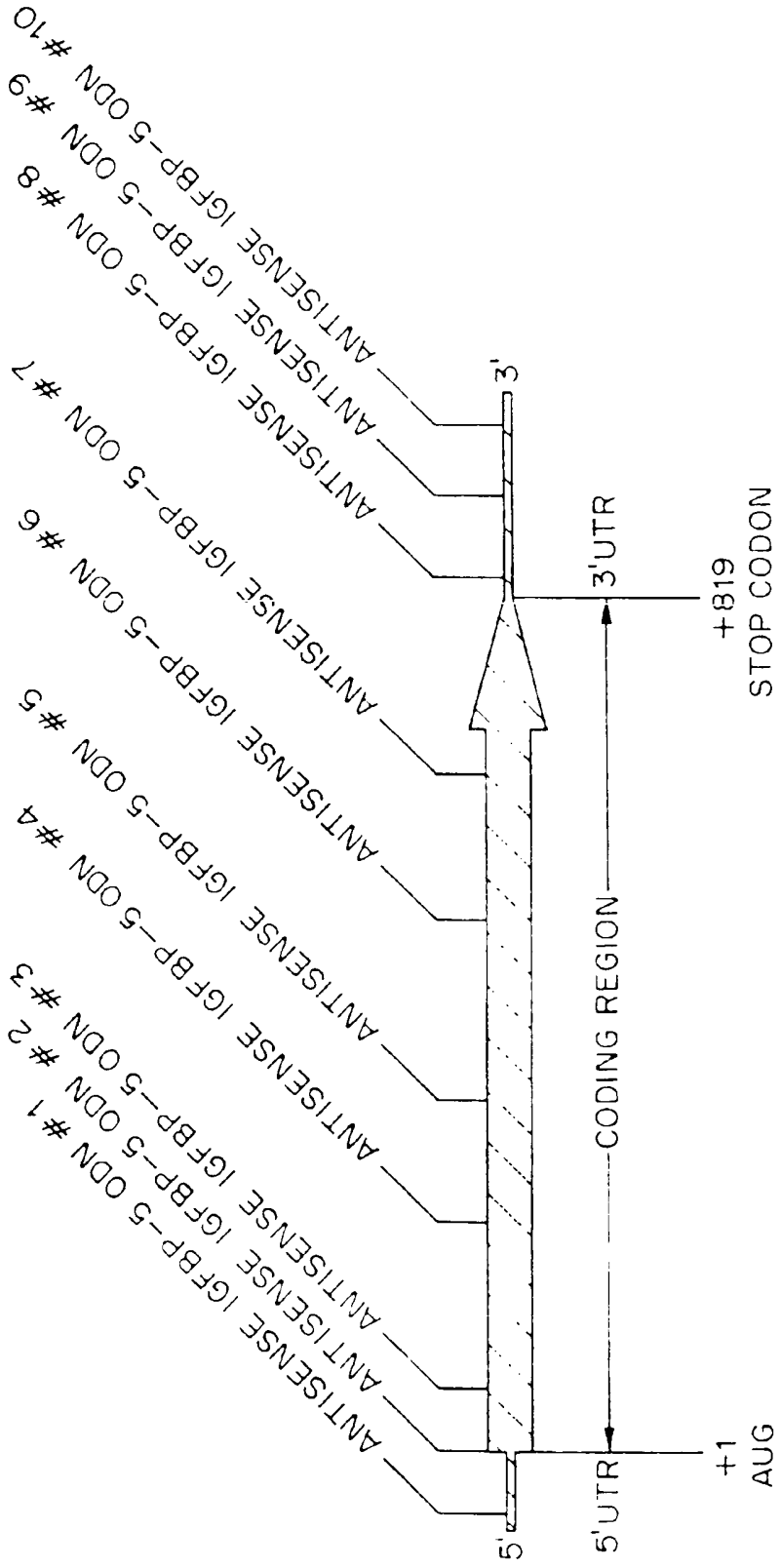

ANTISENSE IGFBP-5 ODN #10
ANTISENSE IGFBP-5 ODN #9
ANTISENSE IGFBP-5 ODN #8
ANTISENSE IGFBP-5 ODN #7
ANTISENSE IGFBP-5 ODN #6
ANTISENSE IGFBP-5 ODN #5
ANTISENSE IGFBP-5 ODN #4
ANTISENSE IGFBP-5 ODN #3
ANTISENSE IGFBP-5 ODN #2
ANTISENSE IGFBP-5 ODN #1
3'
5'
3'UTR
5'UTR
CODING REGION
+819
STOP CODON
+1
AUG

FIG. 9

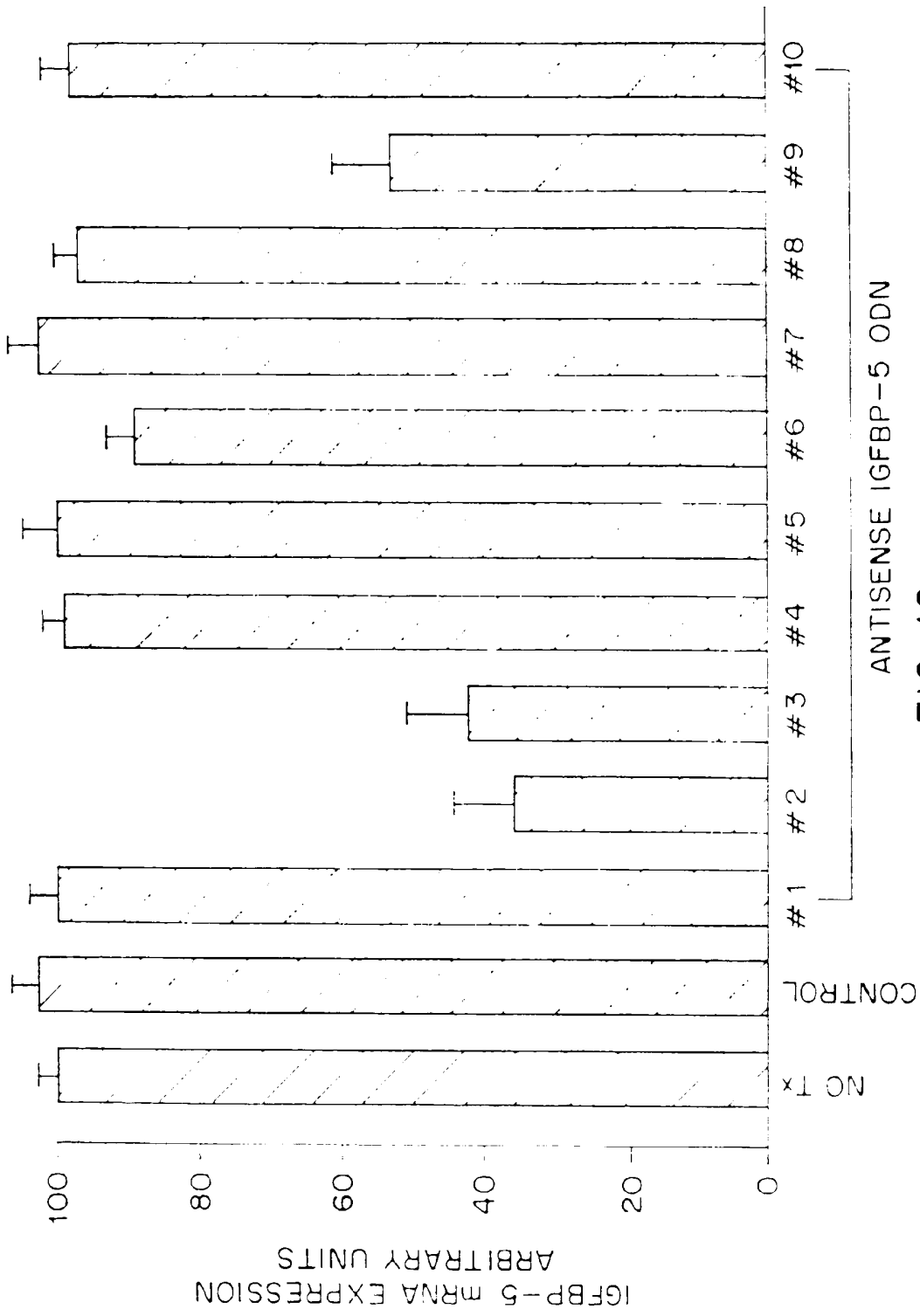

IGFBP-5 mRNA EXPRESSION
ARBITRARY UNITS
100
80
60
40
20
0
NO Tx
CONTROL
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
ANTISENSE IGFBP-5 ODN

FIG. 10

SEQUENCE LISTING

<110> The University of British Columbia
Gleave, Martin
Miyake, Hideaki

<120> Antisense Therapy for Hormone-Regulated Tumors

<130> 80472-8

<140> PCT/CA00/00853
<141> 2000-07-19

<150> US 60/144,495
<151> 1999-07-19

<160> 66

<170> PatentIn version 3.0

<210> 1
<211> 18
<212> DNA
<213> murine

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(18)

<400> 1
gaccacgctg atcaccat 18

<210> 2
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide

<222> (1)..(18)

<400> 2
gtcgcccct ttacctcg 18

<210> 3
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(19)

<400> 3
cgcggtgagc aacaccat 18

<210> 4
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(18)

<400> 4
aggtcatgca gcagcggc 18

<210> 5
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(18)

<400> 5
gctcgcggta gctctttt 18

<210> 6
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(18)

<400> 6
tctttctgcg gtccttct 18

<210> 7
<211> 18
<212> DNA
<213> human

<400> 7
tgcgtgggct ggctttga 18

<210> 8
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(18)

<400> 8
cgtcaacgta ctccatgc 18

<210> 9
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynuclectide
<222> (1)..(18)

<400> 9
agggggtgag ggaaaggt 18

<210> 10
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodecxynucleotide
<222> (1)..(18)

<400> 10
tcaaatagat agatatat 18

<210> 11
<211> 18
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(18)

<400> 11
tacacaaaca cttcctte 18

<210> 12
<211> 18
<212> DNA
<213> murine

<220>
<221> mismatch control
<222> (1)..(18)

<400> 12
gaccacgctc atgaccat 18

<210> 13
<211> 3419
<212> DNA
<213> murine insulin-like growth factor binding protein-5

<400> 13

```
gcagtctctt tggaaacttc taaaagagct aggaaagagc tgcaaagctg tttgggcttt      60
tttccccctt tttgttcctt tttgttaccc cccccctcgg tctgcaccct tctccggact     120
tcacgcagaa cctgcgggtt tcgaagaggt ggtgacagag caggtgttgg ggtccaggtt     180
tggtgaggtc tgggtttttg ccctttttcc cccctcgatt tcaacatttt cccgatcttg     240
ttgtcagccg ccgacgcctc ttacctgttc tgcggcagca gcgcagctgg ccgctgagac     300
cgagcggagt ggggttgcgt tttagatttt aagcaaaggg gggaaaatta agcccaatcc     360
atttttttct tcacctcctc ccttttcaag gcctccaagc taattatttc tgttgctttg     420
gagtgagcaa ttctgtggtt ctctccacca ccacccccaa ttctgacccg atcccgcctg     480
ggggtttcta cggtctccgc tcgctctgcg tgcacctggc gcgcctcttt ttttcacccc     540
caacctgttg caagtcttta atcctcgcaa ttgggacttg cgtgcaggca tctgaatcct     600
ccttgcctca tattttgcaa gtgtttgggg gagagcacct gctctacctg caagagattt     660
aaaaggaaaa aaatctccag gctccctctt tctccacaca ctctcgctct cctgccccgc     720
cccgaggtaa agccagactc cgagaaaatg gtgatcagcg tggtcctcct gctgctggcc     780
gcctatgccg taccggctca aggcctgggt tctttcgtgc actgtgaacc ctgcgacgag     840
aaagctctgt ccatgtgtcc ccccagccct ctgggctgtg agctggtcaa agagcccggc     900
tgtggctgct gcatgacttg cgccctggcg gagggacagt cgtgtggcgt ctacacggag     960
cgctgcgccc agggtttgcg ctgcctcccc cggcaggatg aggagaagcc gctgcacgcc    1020
ctgctgcacg gccgcggggt ttgcctcaac gaaaagagct acggcgagca aaccaagata    1080
agagactctc gggaacacga ggaacccacc acctccgaga tggctgaaga gacctactcc    1140
cccaaggtct tccggcccaa gcacactcgc atttccgagc tgaaggctga ggctgtgaag    1200
aaggaccgca gaaagaagct gacccagtcc aagtttgtgg ggggtgcaga gaacactgcc    1260
caccccagag tcatccctgc acctgagatg agacaggaat ccgaacaagg cccctgccgc    1320
agacacatgg aagcttccct ccaggagttc aaagccagcc cacgcatggt gccccgtgct    1380
gtgtacctgc ccaactgtga ccgcaaagga ttctacaaga gaaagcagtg taagccctcc    1440
cgtggccgca aacgtggcat ctgctggtct ctggacaagt acggaatgaa gctgccgggc    1500
atgcagtacg tggatgggga ctttcagtgc cacgccttcg acagcagtaa cgttttgtta    1560
cgcgtcccct cccttcctcc ccctatccta cccccccagc cccaactccc cccagcgcct    1620
```

cctccaccc caggacgtca ctcatttcat ctcatttagg ggaaatatat atacatatat 1680

atttgagcaa actgaggtcc tcggaatctc tagcaagggc taaggagaca ctccccacca 1740

tgacccccga aatgtattcc tttttgaagc aagttgaacg gacagagaag ggaagggagg 1800

aagaagcaag agggagcgag agatggaaag aaagcaaagc gttggaatag aggaaaagat 1860

ggaaggacag ataggattag agagagaaga gagaaacagc aagggagaaa ggactccaca 1920

accaaggctg aatctgccct tttgctttca gctctagcct ggggtcagaa aaagtgtggc 1980

attcagtgac agccagttta gattggtcaa gggagagaaa gaaacaaggt gtgtcagtgc 2040

ctctcgggtc tgtcccctcc tgcagccagc agtgtggatg gctagacccc tcaccctcct 2100

ctcctcttac ccaagtgcag ggtgatttca tcccccaaatt tacaaagact aaaatgcatt 2160

ccatccctct gaaaataaac aaaagtgagt gattgaagat aggttttccc ccagcagaca 2220

agtgaactca gaatgtgtgc aaatttttact cttgttaaag attttttttaa gaagtcagta 2280

cgcacccccc acactggaaa gacttgattc tccagggtga caagcaattc agaagcgcgt 2340

ggcttcgggc cttgatttca ctagactcaa agctgggccg gcagcctctg tggaggagga 2400

tgagaggtgg agaaaaccaa ggggcttgta ctcacccaca agactccatg tagactttat 2460

aggcatataa atctattttc tttacctttt tttccctttc cctttctttc gaagttttgc 2520

attacctctt taaagtagtt ttttttagga cactgaagat cttcctcatt ctgggaaaaa 2580

tccatatttc acaaatacaa cccagaacgc cagcttggcc tgcgtccagg cagcctttct 2640

cgtgagctac aagtgtggct cttttgtggg gcaccgattt ggatcttctc atgattccaa 2700

acgtgtgttg aagtgaatcc accaagccag gtaactgcca gcacccaagg gtgcatcaag 2760

tgcatagccc aggtcacccc atttcagcct tccaacccgc agaaagtaac tgtctcacac 2820

cacaccacat aaacctgcca gatccatctg taacccactg gcctgcccag acctttttttt 2880

cccatctgca ttttttttttt tgaactgcat tttgaaagcc tccctcagat gccaggctga 2940

cagatcagag agaaactaac atgagagatg acagaggagg aggaagtgga gggtgggggc 3000

agagacttcc acagagagac atagaagatg gagcagaggt ctgggggtgg ggaggacaag 3060

aaagagacag acagaggaaa ataccaatag aattttcttt ggtgtctccc atctaatcaa 3120

ctctctgaga tttcagattg aaaacaattc agggaaagaa cttgaggtag aaatgaggtc 3180

agttcaagtc acagggccca gatggtgggt aactgaggca ggatccaaaa aaaatacttg 3240

tgctttttac tggtgaaaca gattgaaaaa aaaattgaac aacaaaccag tttgtgaaaa 3300

aaaaaaatgg gaaaaaaaat caccccgatg tggaagagct cggctcctct ttagcatttt 3360

ggtacttaag gaaataaaaa agaaaaacct ggaagatctc acattttatt acaaagtga 3419

<210> 14
<211> 1612
<212> DNA
<213> human insulin-like growth factor binding protein-5

<400> 14
ctctcctgcc ccaccccgag gtaaaggggg cgactaagag aagatggtgt tgctcaccgc 60

ggtcctcctg ctgctgccg cctatgcggg gccggcccag agcctgggct ccttcgtgca 120

ctgcgagccc tgcgacgaga aagccctctc catgtgcccc cccagccccc tgggctgcga 180

gctggtcaag gagccgggct gcggctgctg catgacctgc gccctggccg aggggcagtc 240

gtgcggcgtc tacaccgagc gctgcgccca ggggctgcgc tgcctccccc ggcaggacga 300

ggagaagccg ctgcacgccc tgctgcacgg ccgcggggtt tgcctcaacg aaaagagcta 360

ccgcgagcaa gtcaagatcg agagagactc ccgtgagcac gaggagccca ccacctctga 420

gatggccgag gagacctact cccccaagat cttccggccc aaacacaccc gcatctccga 480

gctgaaggct gaagcagtga agaaggaccg cagaaagaag ctgacccagt ccaagtttgt 540

cggggagcc gagaacactg cccacccccg gatcatctct gcacctgaga tgagacagga 600

gtctgagcag ggcccctgcc gcagacacat ggaggcttcc ctgcaggagc tcaaagccag 660

cccacgcatg gtgccccgtg ctgtgtacct gcccaattgt gaccgcaaag gattctacaa 720

gagaaagcag tgcaaacctt cccgtggccg caagcgtggc atctgctggt gcgtggacaa 780

gtacgggatg aagctgccag gcatggagta cgttgacggg gactttcagt gccacacctt 840

cgacagcagc aacgttgagt gatgcgtccc cccccaacct ttccctcacc ccctcccacc 900

cccagccccg actccagcca gcgcctccct ccaccccagg acgccactca tttcatctca 960

tttaagggaa aaatatatat ctatctattt gaggaaactg aggacctcgg aatctctagc 1020

aagggctcaa cttcgaaaat ggcaacaaca gagatgcaaa aagctaaaaa gacacccccc 1080

ccctttaaat ggttttcttt ttgaggcaag ttggatgaac agagaaggga agagaggaag 1140

aagaagagga aaagaaggga aggaagtgtt tgtgtagaag agagagaaag aagaatagag 1200

ttaggaaaag gaagacaagc aggtgggcag gaaggacatg caccgagacc aggcaggggc 1260

ccaactttca cgtccagccc tggcctgggg tcgggagagg tgggcgctag aagatgcagc 1320

ccaggatgtg gcaatcaatg acacttattgg ggtttcccag gatggattgg tcagggcgag 1380

aaaggaaaag gcaaaacact ccaggacctc tcccggatct gtctcctcct ctagccagca 1440

gtatggacag ctggacccct gaacttcctc tcctcttacc tgggcagact gttgtctctc 1500

cccaaattta taaaaactaa aatgcattcc attcctctga aagcaaaaca aattcataat 1560

tgagtgatat taaatagaga ggttttcgga agcagatctg tgaatatgaa at 1612

<210> 15
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 15
ttttttttct tttttcaaaa 20

<210> 16
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 16
atgatagata tatattttc 20

<210> 17
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 17
ccttaaatga gatgaaatga 20

<210> 18
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 18
gtggcgtcct ggggtggagg 20

<210> 19
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 19
gaggcgctgg ctggagtcgg 20

<210> 20
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 20
ggctgggggt gggagggggt 20

<210> 21
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 21
gagggaaagg ttgggggggg 20

<210> 22
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 22
acgcatcact caacattgct 20

<210> 23
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodecxynucleotide
<222> (1)..(20)

<400> 23
acgcatcact caacgttgct 20

<210> 24
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 24
gaaagtcccc gtcaacgtac 20

<210> 25
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodecxynucleotide
<222> (1)..(20)

<400> 25
tccatgcctg gcagcttcat 20

<210> 26
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 26
cccgtacttg tccacgcacc 20

<210> 27
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodecxynucleotide
<222> (1)..(20)

<400> 27
agcagatgcc acgcttgcgg 20

<210> 28
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 28
ccacgggaag gtttgcactg 20

<210> 29
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 29
ctttctcttg atgaatcctt 20

<210> 30
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 30
tgcggtcaca attgggcagg 20

<210> 31
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 31
tacacagcac ggggcaccat 20

<210> 32
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide

<222> (1)..(20)

<400> 32
acgtgggctg gctttgagct 20

<210> 33
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 33
cctccagggg agcctccatg 20

<210> 34
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 34
tgtctgaggc aggggccctg 20

<210> 35
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 35
ctcagactcc tgtctcatct 20

<210> 36
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 36
caggtgcaga gatgatccgg 20

<210> 37

<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 37
gggtgggcag tgttctcggc 20

<210> 38
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 38
tccccgaca aacttggact 20

<210> 39
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 39
gggtcagctt ctttctgcgg 20

<210> 40
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 40
tcattcttca ctgcttcagc 20

<210> 41
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 41
cttcagctcg gagatgcggg 20

<210> 42
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 42
tgtgtttggg ccggaagatc 20

<210> 43
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 43
ttgggggagt aggtctcctc 20

<210> 44
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 44
cgccatctca gaggtggtgg 20

<210> 45
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 45
gctcctcgtg ctcatcggac 20

<210> 46
<211> 20
<212> DNA

<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 46
tctctctcga tcttgacttg 20

<210> 47
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 47
ctccccggtag ctctttttcgt 20

<210> 48
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 48
tgaggcaaaac cccgcggccg 20

<210> 49
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 49
tgcagcaggg cgtgcagcgg 20

<210> 50
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 50
cttctcctcg tcctgccggg 20

<210> 51
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 51
ggagccaggg cagccccctg g 20

<210> 52
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 52
gcgcagcgct cggtgtagac 20

<210> 53
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 53
gcggcacgac tgcccctcgg 20

<210> 54
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 54
cctgggcggc ggtcatccag 20

<210> 55
<211> 20
<212> DNA

<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 55
cagccgcagc cgggctcctt 20

<210> 56
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 56
gaccagctcg cagcccaggg 20

<210> 57
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 57
ggctgggggg gcacatggag 20

<210> 58
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 58
agggctttct cgtcgcaggg 20

<210> 59
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 59
gtcgcagtgc acgaaggagc 20

<210> 60
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 60
ccaggctctg ggccggcccc 20

<210> 61
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 61
gcataggcgg ccagcagcag 20

<210> 62
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 62
gaggaccgcg gtgagcaaca 20

<210> 63
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 63
ccatttctcc ttagtgcccc 20

<210> 64
<211> 20
<212> DNA

<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 64
cctttaccac ggggtggggc 20

<210> 65
<211> 20
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(20)

<400> 65
aggagagcga gagtgcaggg 20

<210> 66
<211> 21
<212> DNA
<213> human

<220>
<221> antisense IGFBP-5 oligodeoxynucleotide
<222> (1)..(21)

<400> 66
gaccgcggtg agcaacacca t 21